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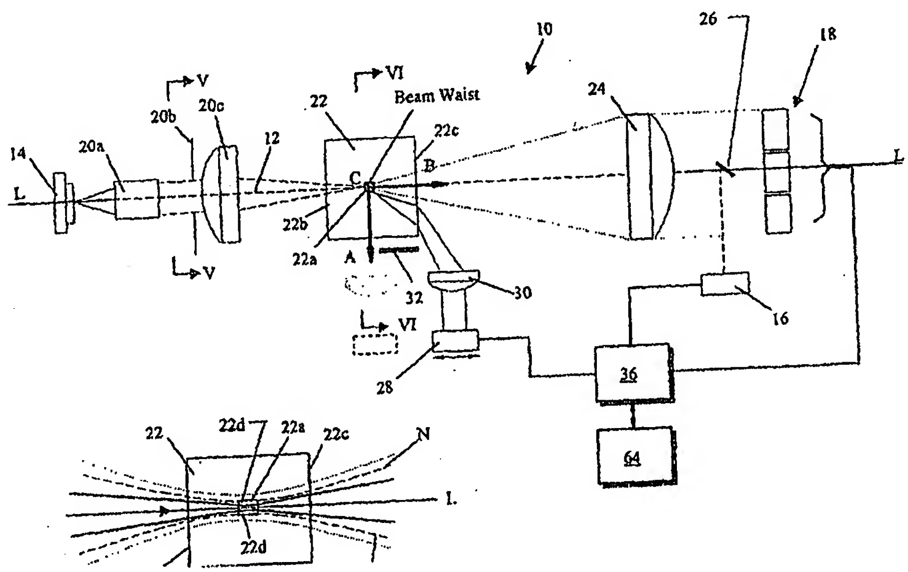
(54) Title: OPTICAL DEVICE AND METHOD FOR MULTI-ANGLE LASER LIGHT SCATTER



(57) Abstract: The present invention relates to an optical system for an apparatus for multi-part differential particle discrimination to facilitate analysis, classification, and sorting of various fluid components for presentation. The optical system is characterized by one or more of the following: a synchronized illumination beam and flow cell conduit, a flow cell arrangement to control back reflection, and light sensor arrangement to particularly gather a specific range of light scatter, such specific range of light scatter directly corresponding to at least one type of particle capable of being identified by the apparatus.

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INTERNATIONAL SEARCH REPORT

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3 701 620 A (BERKMAN RICHARD M ET AL) 31 October 1972 (1972-10-31) abstract; figures 6,7 column 4, line 16-29 column 4, line 56 -column 5, line 27 ---	1,2,5,8, 11
X	US 3 561 874 A (WEBER ROBERT J) 9 February 1971 (1971-02-09) abstract; figures 1,2 column 1, line 69 -column 2, line 13 column 3, line 69 -column 4, line 14 ---	1,2,5, 10,11,26

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 3701620	A	31-10-1972	NONE		
US 3561874	A	09-02-1971	NONE		
US 4565446	A	21-01-1986	EP	0104661 A2	04-04-1984
US 5061065	A	29-10-1991	WO	9105995 A1	02-05-1991
US 4192614	A	11-03-1980	DE	2604302 A1	19-08-1976
			GB	1515632 A	28-06-1978
			SE	7601242 A	09-08-1976
			SE	7906667 A	09-08-1979
EP 0168939	A	22-01-1986	EP	0168939 A2	22-01-1986
EP 0527652	A	17-02-1993	US	5235409 A	10-08-1993
			EP	0527652 A2	17-02-1993
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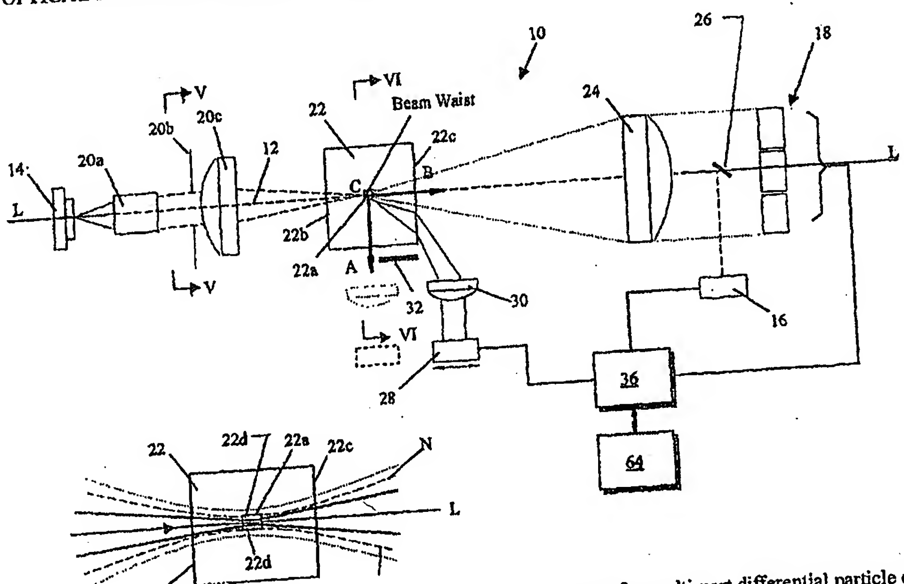
(54) Title: OPTICAL DEVICE AND METHOD FOR MULTI-ANGLE LASER LIGHT SCATTER



(57) Abstract: The present invention relates to an optical system for an apparatus for multi-part differential particle discrimination to facilitate analysis, classification, and sorting of various fluid components for presentation. The optical system is characterized by one or more of the following: a synchronized illumination beam and flow cell conduit, a flow cell arrangement to control back reflection, and light sensor arrangement to particularly gather a specific range of light scatter, such specific range of light scatter directly corresponding to at least one type of particle capable of being identified by the apparatus.

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in relation to known statistical sampling errors, an inadequate number of micro-particles could be analyzed by microscopy and only extreme pathological deviations were intercepted reliably. On the other hand, by 1930 R.A. Fisher had taught that, if a sufficient number of well-chosen, different, robust measurements were made on complex organic entities such as cells during an investigation, then, it would be straightforward to objectively assign the different tested elements to appropriate natural sets and subsets.

From the 1930's onwards attempts were made to automate the time-consuming number-accumulation chore and classification challenge of cell and micro-particle analysis. For clinical cell analysis, these endeavors evolved along two main paths: (i) image analysis microscopy, which examines all microscopic objects, and (ii) flow analysis, or generalized flow cytometry of micron-sized and of sub-micron particles down to macromolecules.

In classical microscopy a large specimen is placed into an object plane which is orthogonal to the optic axis of the microscope. That classical plane is occupied by a vast expanse of sample material which is illuminated over a wide field, i.e., a field much larger in area (and frequently also in depth) than the arbitrarily definable voxel (or volume element or "cell of space") occupied, for example, by a small single tissue cell or by an ensemble of granules within, perhaps, an eosinophil cell or a suspension or solution in a spectrophotometric cuvette. In whatever manner the voxel (or "cell") is defined, there is a very vast number of these illuminated elements in the classical microscopic object. Each such illuminated voxel of such a sample preparation causes the scattering (or, in certain settings fluorescence) of light. In relation to the signal light from a specific voxel of interest, the light from all the other structures in the microscopic field represents signal-degrading background noise. That background noise light can easily exceed 99.9% of the total light received in the microscope's image location of the specific voxel. Hence, significant detail relating to such classically viewed voxels is likely lost to the viewer.

"Confocal" microscopy is an automated technique for avoiding the degrading effects of that frequently overwhelming background noise discussed above. The background is avoided by contemporaneously having both the illumination lens system(s) and the interrogation lens system(s) focus essentially on one voxel at a time. Additionally, computer techniques are used to synthesize three-dimensional high-signal images whose rich information content could not

particulate-containing fluid for interrogation in flow-through cells rather than merely in static cells or cuvettes.

When lasers were introduced, MALS could more appropriately be termed MALLS (multi-angle laser light scatter); and in the 1960's many scientists incorporated lasers into the earlier Crosland-Taylor flow cytometry systems. Thereby, additional optic sensing axes could be placed into the traditional orthogonal microscopic object plane which had been co-opted by flow cytometry for the particle monofile; and the illuminating laser pencil for flow cytometry could also reside in that plane. Indeed, many in the art perceived that an interrogation apparatus could be arranged with laser and particle filament intersecting at the optic scattering and fluorescence origin of a spherical radiation domain. Crosland-Taylor had already introduced bubble-free degassed carrying liquids. Therefore, except for the thin sample fluid filament carrying the test particulates through a finite illuminated radiation centroid, this spherical scattering space was occupied only by optically clear fluid in which the illuminating laser pencil need not leave a trace.

Such system enabled rapid MALLS examination of stained or unstained particles ranging in size from protein molecules through bacteria and mammalian cell organelles to large organic cells. Additionally, non-optic sensors such as electrical impedance and capacitance cell interrogation principles could be applied to each larger particle, whether concurrently with an optical interrogation or in exact tandem synchronization--of a type which had evolved in the flow cytometry subfield of cell sorting (e.g., U.S. patents 3,710,933 and 3,989,381).

By the 1970's flow cytometry could measure a host of both "intrinsic" and "extrinsic" particle properties. The so-called "intrinsic properties" can be documented without the use of special reagents while "extrinsic" properties are elicited via physical, chemical or biological reagents such as altered pH or tonicity and/or dyes and/or coupled monoclonal antibodies or tags.

FIGS. 1A and 1B summarize the state of flow analysis transducers in the 1970's. These figures were based on work performed to produce vortex-assisted, super-focused, low-variance, low-noise, many-centimeter-long cell monofiles. In particular reference to the "tank" of FIG. 1A, U.S. patent 5,138,181 teaches that even without induction of a vortex "the fact

filtered out from the longer emitted-wavelength measurements of these systems. With respect to fluorescent particle interrogations, these systems effectively reduce to that illustrated in FIG. 1C and do confocal fluorescent flow cytometry.

In the early 1970's one of the desires of flow cytometry became recognition of "intrinsic" optic scatter of sub-microscopic structures such as bacteria, organelles and characteristics of small cells such as platelets. During this time, MALS explorations using helium-neon laser and argon ion laser Biophysics Cytograph systems with both circular and square cross-sections were conducted. However, when looking for the sub-microscopic scatter substructures known from electron microscopy (as opposed to also known fluorescent sub-micron substructures) it became evident that, despite the great improvements in hydrodynamic focusing made available per U.S. patent 3,871,770, there was a fundamental problem of signal-degrading optic scatter background noise in affordable walled transducer derivatives (e.g., FIG. 1B).

FIG. 1C shows diagrammatically that, in the wall-illumination-avoiding setting, micro-particles such as bacteria or cellular granules or macromolecules, do generate highly informative MALLS signatures which can be sampled over almost 4π steradians about any angle outside the intense 13° narrow-angle forward scatter cone.

Of course, the micro-particle illustrated in Fig 1D also generates the same unique MALLS signals in the wall-illumination-degraded conduit structures. Unfortunately, using any of the available conventional flow cytometry illumination arrangements, the walls of these walled-conduit structures conventionally generates such an enormous quantity of background scatter noise, that, for practical purposes, the unique MALLS signatures from sub-micron structures can not be usefully discriminated therefrom. The improved positional, orientational and temporal variance that was enabled by spiral hydrodynamic focusing to overcome excitation field inhomogeneities was totally wasted in the optic scatter setting of walled-conduit flow cuvettes.

By the 1980's Crosland-Taylor's optic transducer flow cells had undergone numerous modifications and design variations (see, for example, U.S. patent 3,661,460). However, in each typical flow cell there had to be a physical boundary between the fluid medium carrying the particles and the media through which the illuminating light entered and left the fluid in the

some, theoretically and practically, to give the right discriminations; provided however, wall-illumination-avoiding analytical conditions are used.

Recapitulating, FIG. 1A shows a conventional wall-illumination-avoiding optic "tank". In such an arrangement, hydrodynamically focused particulates generally pass from an impedance and /or capacitance or RF sensor into a fluid body which is so vast in relation to a particle file interrogated by a laser beam that optic irregularities at the very distant walls through which the laser enters and exits the fluid can be arranged to contribute mathematically negligible background noise to appropriate scatter signals gathered as in confocal microscopy at the illuminated center of a vast conceptual spherical radiation domain. FIG. 1B shows a wall-illumination-degraded optic "conduit". In such a construction, hydrodynamically focused particulates generally pass from an impedance and /or capacitance or RF sensor into a transparent walled conduit flow cell. In such a typical clinical flow cytometry arrangement, the interrogated particle file is so close to the optically irregular flow cell walls that optic background noise scattered from those walls or edges overwhelms scatter signals from sub-micron particles. This happens not only when the conduit cross section is circular but also when that cross section is square or rectangular, for example, the cost-effective 250μm x 250μm (flow path dimensions), polished flow cells (Part No. 131.050-QS, Hellma, Corp.) which have been routinely available since the 1980's.

This signal/noise scatter problem in walled-conduit flow cells was summarized in U.S. patent 4,515,274. The reference provides that, "the smaller the size of the flow cell 16, the better its optical characteristics, in that the flow cell approaches a point source for optical signals." As can be seen from this rationale, it is not conventionally the biological cell or micro-particle that is considered as the point source for the harvested optical signals, but the more than 10,000-fold larger illuminated flow cell. Unquestionably, the scatter signal from a biologic cell only rises with difficulty above the optic noise produced from such conventional systems. For more modern systems, this issue remains unresolved.

As opposed to addressing the generation of such background noise, enormous masking efforts and diffraction blocking is practiced in/by conventional systems to get the signal at the advocated and implemented single intermediate "MALS" scatter sensor to rise above the prevalent scatter noise. By contrast, in "tanks" without a wall-proximity and without that

beam-conduit interaction, to facilitate reliable application of the common mode rejection ratio technique.

SUMMARY OF THE INVENTION

At least one aspect of the present invention is drawn to a particle analyzing apparatus for discriminating particulate element(s) in a fluid flow for purposes of analysis, classification, sorting, and presentation. The apparatus has an illumination source for emitting an illumination beam, a flow cell, and a light sensor. The flow cell has: a flow passage, through which a fluid flow containing particles can pass, a first exterior face perpendicular to a first axis of the flow cell, and a second exterior face perpendicular to a second axis of the flow cell. Of note, the first axis is substantially co-axial with an illumination beam emitted from the illumination source, and the first exterior face and the second exterior face are orthogonal. The light sensor, oriented substantially parallel to the second exterior face of the flow cell and displaced a sufficient distance from the second axis to enable receipt of a prescribed range of light passed by the first exterior face, operatively receives light from the illumination source via the flow cell.

Another aspect of the present invention is directed to a particle analyzing apparatus for discriminating particulate element(s) in a fluid flow for purposes of analysis, classification, sorting, and presentation. The apparatus includes an illumination source to emit an illumination beam, a flow cell, a sample supply, and a first light sensor. The flow cell has: a flow passage extending through the flow cell, a rear surface portion that receives an illumination beam emitted by the illumination source, and a forward surface portion that passes light formed from an illumination beam received by the rear surface portion. The flow cell has a first axis, which is substantially co-axial with an axis of an illumination beam emitted from the illumination source, and a second axis, which is orthogonal to the first axis. The sample supply operates to introduce a fluid to the flow passage of the flow cell. The first light sensor operates to receive at least light passed from the flow cell in response to an interaction with an illumination beam emitted by the illumination source and a fluid supplied to the flow passage of the flow cell. A light receiving surface of the light sensor is substantially parallel to the axis

illumination beam, a flow cell, a sample supply, a focusing optical system, and a light sensor. The flow cell has a flow passage extending through the flow cell, a rear surface portion that receives an illumination beam emitted by the illumination source, and a forward surface portion that passes light formed from an illumination beam received by the rear surface portion. The sample supply functions to introduce a fluid to the flow passage of the flow cell. The focusing optical system, positioned between the illumination source and the flow cell and having an aperture, forms and positions a beam waist of an emitted illumination beam relative to the flow passage. The first light sensor operates to receive light passed by the flow cell. The illumination beam source emits an illumination beam having an intensity profile characterized by a central, principal intensity peak that originates and terminates at substantially null intensity values. With respect to a synchronized relationship of elements of the system, the flow cell is further arranged relative to the illumination source so that the principal intensity peak is substantially centered with respect to the flow path, and such illumination beam intercepts internal boundaries of the flow cell, which define the flow path, proximate to the null intensity values.

An object of the present invention is to provide an optical system having a synchronized illumination beam and flow cell.

Another object of the present invention is to provide an optical system having a flow cell oriented to control back reflection when subjected to an illumination beam.

Another object of the present invention is to provide a particle analyzing apparatus, to discriminate particles in a fluid flow for analysis, classification, sorting, and presentation, having a flow cell-light sensor relationship to enable a gathering of a specific range of light scatter passed by the flow cell, such specific range of light scatter directly corresponding to at least one type of particle capable of being identified.

Another object of the present invention is address the objects of this invention, whether individually or in combination, and maintain an appropriate overall apparatus size.

Other objects and advantages of the present invention will be apparent to those of ordinary skill in the art having reference to the following specification together with the drawings

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Various embodiments, including preferred embodiments, are described in detail below with reference to the drawings.

FIG. 2A illustrates a particulate element (or particle) discrimination apparatus 10 in accordance with the present invention. The apparatus 10 is effectively defined along a path 12 (or axis-L) that extends between a laser source 14 and a plurality of light scatter sensors 16 and 18.

The laser source 14 can be any one of a plurality of conventional lasers suitable for this application, for example, a low power helium-neon laser. In a preferred embodiment, the laser source 14 is a solid-state laser diode.

A laser beam emitted from the laser source 14 passes through a focusing optical system 20, e.g., a collimating lens 20a, an aperture structure 20b, and a focusing lens 20c, before reaching a flow cell 22. This laser path is in reference to the illustrated axis-A and axis-B as well as an axis-C', which is substantially normal to the page illustrating FIG. 2A, that corresponds to a fluidic center of the flow cell 22.

As illustrated, the flow cell 22 includes a flow path 22a, in accordance with axis-C', in which flows a hydrodynamically focused fluid filament that contains particulate elements to be discriminated. The flow path 22a is in fluid communication with a fluid source 23, which typically holds a diluted sample for delivery to the flow cell 22 (FIG. 3). The flow cell 22 is formed of an optically transparent material, for example, quartz in the form of strain-free fused silica, or the like. Although the flow cell 22 could take a partially arcuate form, a preferred form of the flow cell 22 is one of the readily available flow cytometry flow cuvettes having parallel sides. In particular, the flow cell 22 has a square shape with a complementary square-shaped flow path 22a. However, the perimeter shape of the flow path 22a is not optically dictated by the exterior shape of the flow cell 22. Accordingly, irrespective of the exterior shape of the flow cell 22, the flow path 22a of the flow cell 22 can assume a rectangular or other shape conducive to maintaining a position of a particulate element carrying fluid filament, which flows therethrough, relative to an illumination beam as well as at least those walls of the flow path 22a that are parallel to axis-B of FIG. 2A.

sensor. Further yet, the structure of element 26 could alternatively be a receiving face of an optical fiber, which would function to capture a specific range of light scatter and direct it to a corresponding light sensor.

In FIG. 2A, an illustrated light sensor 28 is positioned relative to the flow cell 22 to receive a particular range of light scatter output from the flow cell 22. It is intended that this particular range of MALLS light scatter provide information not otherwise cost-effectively discernable from the approximately 360 degree MALLS light scatter sensors 16 and 18.

When using the apparatus 10 as a hematology analyzer, for example, this particular range can specifically relate to abundant sub-micron granules in particular subpopulations of biological cells (e.g., eosinophillic granulocytes) to be discriminated. Accordingly, while the particular range of light depends on the characteristics of the particle, in a preferred embodiment, the light sensor 28 receives a range of light scatter from 25°-50° (such angle being with respect to an axis of the flow cell 22). In a more preferred embodiment, the light sensor 28 receives a range of light scatter from 30°-45°. In a most preferred embodiment, the light sensor 28 receives a range of light scatter from 35°-43°. The illustrated position of the light sensor 28 is characterized by its light receiving surface being substantially parallel to a surface of the flow cell 22, such surface being effectively perpendicular to the axis-A.

In regard to application of the common mode rejection ratio technique, as discussed in the background section of this disclosure, the light sensor 28 can appreciably provide at least first and second robust and light scatter. The basis of the implicit common mode compensation in a two-angle design is that, if appreciable systematic background scattering error still occurs for the first sensor 18 (for example, at 13°) and the sensor 28 (for example, at 37°), then a reasonably-constant-function background error will be introduced into both of the outputs of sensors 18 and 28 and these errors should leave any cell cluster position contrasts in related, generated biplot scattergrams reasonably unaffected. Thus, real differences between, for example, the sub-micron granules of such granulocytes as neutrophils, eosinophils, and basophils, should be far more apparent than if only a single scatter measurement is resorted to at any one of the MALLS locations between 13° and 90°.

At each edge of the flow cell 22, it is possible to specify two very exact angular locations. For example, at the 45° corner of a square flow cell (e.g., FIG. 2A), the 37°

readily be transposed, together with mirror imaging at each corner, to any of the four corners of the flow cell shown in Fig 2A. One such example of a transposition is shown in Fig 2C.

FIG. 2D illustrates a structural arrangement adapted to collect a particular sub-range of light scatter with an additional forward light sensor array 18a. For such arrangement, it is necessary to position a collection lens 24' very near to an exiting face of the flow cell 22. Irrespective of the position of the collection lens 24' relative to the flow cell 22, such collection lens 24' must have a dimension greater than the collection lens 24 of FIG. 1A. Consequently, increasing the size of the collection lens 24 can undesirably increase both costs and a physical size of the apparatus 10.

FIG. 2E illustrates a structural arrangement adapted to collect the desired sub-range of light scatter with one or more light sensors 28' arranged with respect to the corners of the flow cell 22. Prior to the light sensors 28', a structure is provided that includes a material 30a', having a refractive index substantially equal to that of the flow cell material, and a glass cover 30b'. While such structure is suited for collecting the prescribed light scatter, the structural relationship between the flow cell 22 and the sensor 28' is more complex than utilization of the corner relationship of sensor 28 in FIG. 2A (or FIG. 2C), thus requiring greater accuracy in placement and more elaborate constructions in manufacturing to minimize angular detection differences between instruments.

As mentioned above, the laser source 14 is preferably a solid-state laser diode. Using a laser diode enables the optical path between the laser source 14 and the flow cell 22 to be significantly shortened in comparison to the minimum focal distance needed for other sufficient laser sources, e.g., a helium-neon laser. Thus, the overall size of the apparatus 10 can be made smaller.

In order to obtain the most accurate light scatter results, it is important to obtain a maximum signal-to-noise ratio, in the broad, conventional sense. In a more specific, narrower technical sense, this corresponds to minimizing detection of limiting background light and maximizing a signal-to-noise ratio. "Signal" is defined as a "net signal," i.e., a gross mean signal minus mean background signal, and metrologic "noise" is defined narrowly as the root mean square fluctuation of the gross signal about the mean gross signal.

discussed in greater detail below, such slight rotation is not incompatible with avoiding flow cell edge illumination and thus avoiding its associated light scatter.

While the flow cell 22 can receive an anti-reflective coating, to avoid the uncertainty and expense of such coatings, the flow cell 22 is preferably uncoated. To this end, an uncoated, polished face of the flow cell 22, which does not include the edges of such face, reflects approximately 4% of an approximately orthogonally received light at each glass-air interface.

Additional light can be reflected from the internal flow cell surfaces and even from the hydrodynamically focused particulate filament if there are certain differences between sample and sheath streams. If even a small portion of this reflected light were to return to the laser source 14, it would substantially increase the likelihood of light fluctuations and beam noise.

Because of the angular displacement of the flow cell 22 of FIG. 3, back light reflection is not received by the laser source 14. In accord with the illustrated example, the 3° tilt of the flow cell 22 causes the centerline of the reflected beams to be approximately 6° from the axis-L at any single reflection surface. Since the extent of the beam in the vertical direction is less than 3°, the closest (i.e., the illustrated lowest) ray from the reflected beam will be greater than 3° away from the highest ray of the illuminating beam. As shown, a beam dump 34 is preferably provided to extinguish any back reflections by the flow cell 22.

Critical to obtaining repeatable discrimination results from the detected light scatter, it is very important that the illumination of each particle be both constant and consistent. To achieve this, each particle must pass through the illumination beam (i.e., laser beam) as close to the mean path as possible, and the light intensity must be as near uniform as possible in the area of the mean path.

As emphasized in FIGS. 1A-1D, maintaining a course for each particle along a constant narrow flow path 22a is accomplished by hydrodynamically focusing the sample using a surrounding particle-free sheath fluid. The method of U.S. Patent 3,871,770 was an improvement over long-known earlier methods. These earlier methods suffice to provide excellent hydrodynamic flow stability in a walled conduit of the preferred embodiment, especially if both sample and sheath are driven by positive displacement pressure as by separate syringes. Use of such known methods for the present invention maintains the course

the page, with the illustrated hatching of each spot suggesting the relative density of the peak symbolized by that spot. Thus, the doubly-hatched central peak characterizes the most intense illumination while the peripheral peaks are much less intense.

FIG. 4 shows this light intensity relationship in another way. FIG. 4 can be conceived as a section through the three central intensity peaks of FIG. 6. Specifically, ignoring the materials and refractions at the flow cell 22 itself, the intensity profile of the illumination beam in accordance with the present invention can be written as:

$$I = \{\sin(\pi \cdot W \cdot x/\lambda \cdot f)/(\pi \cdot W \cdot x/\lambda \cdot f)\}^2 \qquad \text{Equ. (1)}$$

where, x represents the distance value corresponding to an optical spot size formed by an illumination beam emitted from the illumination source, λ represents a wavelength of such illumination beam, f represents the focal length of the focusing subsystem, and W is a width dimension of the aperture of the aperture structure 20b (FIG. 5). A plot of this intensity profile is shown in FIG. 4. In reference to this figure, the ordinate conveys an intensity value in relation to a relative cross-section dimension of an optical spot.

In appreciation of Equ. (1), the dimensions of a beam profile are accurately and reliably determined by two optical parameters of the system: a focal ratio (or *F*-number) and a wavelength of the illumination beam. The *F*-number is a ratio of the focal length of the focusing optical system 20 to the width dimension of the aperture of the aperture structure 20b.

In reference to the aperture structure 20b of FIG. 5, it is preferred that its aperture be of a rectangular form. While other aperture shapes (e.g., circular, elliptical, square, etc.) are permissible and would enable proper functionality of the present invention, a rectangular shape conventionally allows independent control of the *F*-number parameters in both horizontal and vertical directions.

As an example of one possible configuration of the present invention, the longitudinal dimension of a rectangular aperture, as illustrated in FIG. 5, is approximately 0.635 mm, which results in a corresponding focal ratio of approximately 157:1 and a spot size of approximately 100 μm. In general accordance with the plot of FIG. 4, the intensity at 125 μm from the center of the beam center is only about 3.2% of the maximum intensity of the illumination beam.

illumination beam and a sub-micron particle passing through the flow path 22a. As intense background light scatter reduces the ability of a system to discern valuable information from the net signal MALLS light scatter, some devices have employed elaborate screening and rejection methods against tolerated signal degrading walled-conduit light scatter (see U.S. Patent 4,577,964 and U.S. Patent 5,125,737). Although the approach in U.S. Patent 4,577,964 did permit sensitive optic counting of human platelets and U.S. Patent 5,125,737 patent does provide certain light scatter information for large human leukocytes at a selected light scatter locus via a walled conduit scatter sensor of the type of Fig 1D, neither approach, which tolerates very high non-specific background scatter, can be a basis for general MALLS interrogation of sub-micron particles, as these particles are below the optic scatter detection threshold of these scattering systems examples.

In accordance with the present invention, the above background scatter problems of the conventional art are avoided. In particular, the focal ratio of the lens 20c positions a center of the principal intensity peak (P1) of the illumination beam profile at a point approximately in a center of the flow path 22a with respect to the width dimension between the internal side walls of the flow cell. Moreover, the nulls (B1) are arranged to proximally coincide with the internal boundaries/corners 22d of the flow path 22a, thus the intense walled-conduit background light scatter of FIG. 1D is minimized. Of note, such positioning can be achieved even if the approximately straight walls of the flow path 22a are not totally parallel to the axis B of Fig 2A. To better illustrate the "synfocal" principle of this synchronized focusing aspect of the present invention, reference is made to FIG. 7.

Here particles flow in a hydrodynamically focused, centralized fluid filament through the flow cell 22 from bottom to top. The illumination beam, being normal to the page on which the illustration is made, is shown in cross-section. This beam intercepts the flow cell 22 and illuminates particles as they flow centrally through the flow cell 22.

The energy profile of FIG. 4 is shown below the flow cell 22 section to exhibit the synchronization of the laser beam null intensities B1 with the scattering walls and edges 22d of the flow cell 22. This nadir or bottom B1 of the energy profile is also illustrated in FIG. 2B as path N.

58, and 60 to predetermined values that are individually representative of specific subpopulation members of biological cells. Output from the comparators can be counted, for preparation of histograms or matrices, or combined in at least two-member groupings, for purposes of preparing scattergrams. Output from the analyzer 62 is supplied to a display device 64 (e.g., printer, CRT, etc.).

Of note, many types of known additional particle interrogation mechanisms (and applications, such as cell sorting) were discussed in the background portion of this disclosure. Though incorporated by reference, those not benefiting directly from our invention(s) to reduce MALLS background scatter and laser noise are not displayed in the figures of our teachings. However, the different aspects of the present invention individually and jointly enable cost-effective SMALLS analysis in many settings and for many different applications and may be equally applied to such.

While the invention has been described herein relative to a number of particularized embodiments, it is understood that modifications of, and alternatives to, these embodiments, such modifications and alternatives realizing the advantages and benefits of this invention, will be apparent to those of ordinary skill in the art having reference to this specification and its drawings. It is contemplated that such modifications and alternatives are within the scope of this invention as subsequently claimed herein, and it is intended that the scope of this invention claimed herein be limited only by the broadest interpretation of the appended claims to which the inventors are legally entitled.

5. An apparatus in accordance with Claim 1, wherein the flow cell is not orthogonal to an illumination beam emitted by the illumination source.

6. An apparatus in accordance with Claim 3, further comprising a focusing optical system, positioned between the illumination source and the flow cell, to form and to position a beam waist of an emitted illumination beam within the flow passage.

7. An apparatus in accordance with Claim 6, further comprising a collection optical system, positioned between the flow cell and the light sensor, to collect light passed by the flow cell and further pass such light to the light sensor.

8. An apparatus in accordance with Claim 1, wherein the flow cell is displaced by an angle relative to a direction orthogonal to the optical axis, and the angle is between 2.5°-10°.

9. An apparatus in accordance with Claim 1, further comprising a reflective surface to intercept and redirect in direction a component of at least light passed from the flow cell in response to an interaction with an illumination beam emitted by the illumination source and a fluid supplied to the flow path of the flow cell, and a light sensor to receive the component.

10. An apparatus in accordance with Claim 1, wherein the portion of the exterior surface to receive an emitted illumination beam from the illumination source does not have an anti-reflective coating.

11. An optical system for a particle analyzing apparatus for discriminating particulate element(s) in a fluid flow for purposes of analysis, classification, sorting, and presentation, the apparatus having an illumination source for emitting an illumination beam and at least one light sensor to receive at least a light component of an emitted illumination beam, the system comprising:

a focusing optical system, positioned along an optical axis of the system, to receive an

a sample supply to introduce a fluid containing particles to the flow passage of the flow cell;

a focusing optical system, positioned between the illumination source and the flow cell and having an aperture, to form and to position a beam waist of an emitted illumination beam relative to the flow passage; and

a first light sensor to receive light passed by the flow cell,

wherein an emitted illumination beam has an intensity profile characterized by a central, principal intensity peak that originates and terminates at substantially null intensities, and

wherein the flow cell is further arranged relative to the illumination source so that the principal intensity peak is substantially centered with respect to the flow passage, and such illumination beam intercepts, proximate to the null intensities, internal lateral boundaries of the flow cell, which define at least in part the flow passage..

17. An apparatus in accordance with Claim 16, wherein the flow cell has a first axis, which is substantially co-axial with an optical axis of the apparatus, and a second axis, which is orthogonal to the first axis.

18. An apparatus in accordance with Claim 17, wherein a light receiving surface of the first light sensor is substantially parallel to the axis of an illumination beam emitted from the illumination source, and the first light sensor is displaced a prescribed distance from the second axis.

19. An apparatus in accordance with Claim 18, wherein the first light sensor receives a specific range of light passed from the flow cell in response to an interaction with an illumination beam emitted by the illumination source and a fluid supplied to the flow passage of the flow cell, such specific range extending between at least 30° and no greater than 45° relative to the first axis.

comprising:

an illumination source to emit an illumination beam along an optical axis of the apparatus;

a flow cell, positioned along the optical axis, having: a flow path extending through the flow cell and a portion of an exterior surface to receive an illumination beam emitted from the illumination source;

a sample supply to introduce a fluid to the flow path of the flow cell; and

at least one light sensor, positioned along an optical path of the apparatus, to receive at least light passed from the flow cell in response to an interaction with an illumination beam emitted by the illumination source and a fluid supplied to the flow passage of the flow cell,

wherein the flow passage has a longitudinal axis, and

wherein the longitudinal axis of the flow passage is angularly displaced from an axis perpendicular to the optical axis so that the flow cell is oriented to prevent a back light reflection that is parallel to the optical axis in response to an emission of an illumination beam from the illumination source.

27. A method for optically differentiating at least one type of particulate element carried by a fluid filament, the method comprising the steps of:

emitting a laser beam from a laser source along an optical path and through a flow cell having an internal passageway that receives the fluid filament;

sensing at least a portion of light scatter produced by an interaction between a particulate element, carried by the fluid filament, and the laser beam; and

analyzing sensed light scatter to identify the particulate element,

wherein internal boundaries define the internal passageway of the flow cell, and

wherein the flow cell is arranged relative to the optical path so that minor intensity regions of the laser beam are positioned proximate to the internal boundaries.

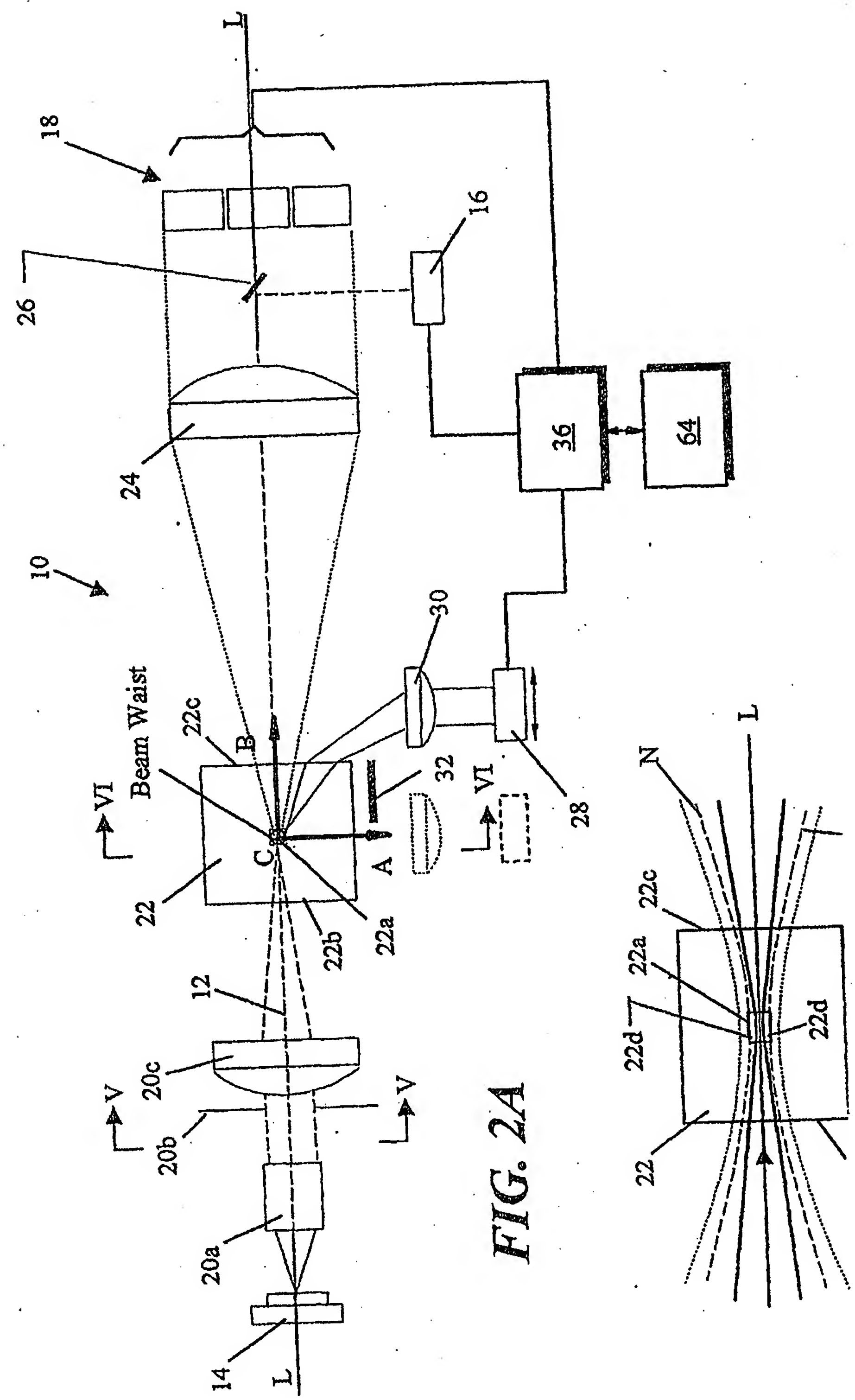
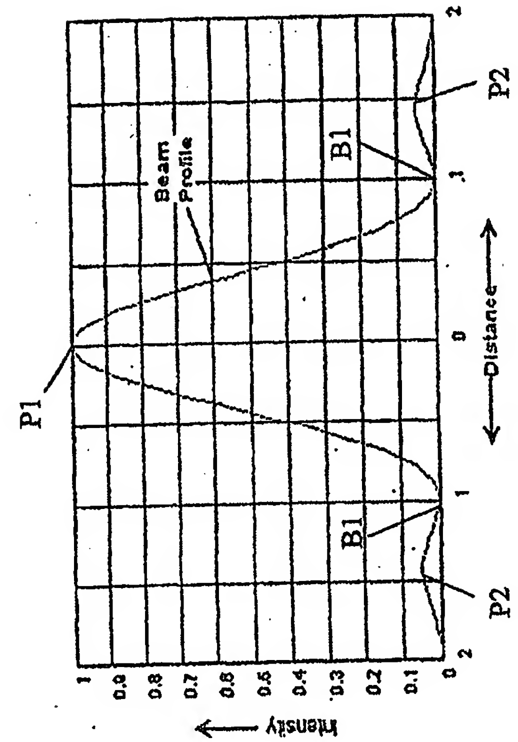

L
18
26
16
24
36
64
10
30
Beam Waist
22c
B
VI
32
C
A
22
22b
22a
28
12
20c
V
20b
20a
14
N
22d

FIG. 2A

FIG. 4


Intensity
Distance
Beam Profile
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P2
B1
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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
1
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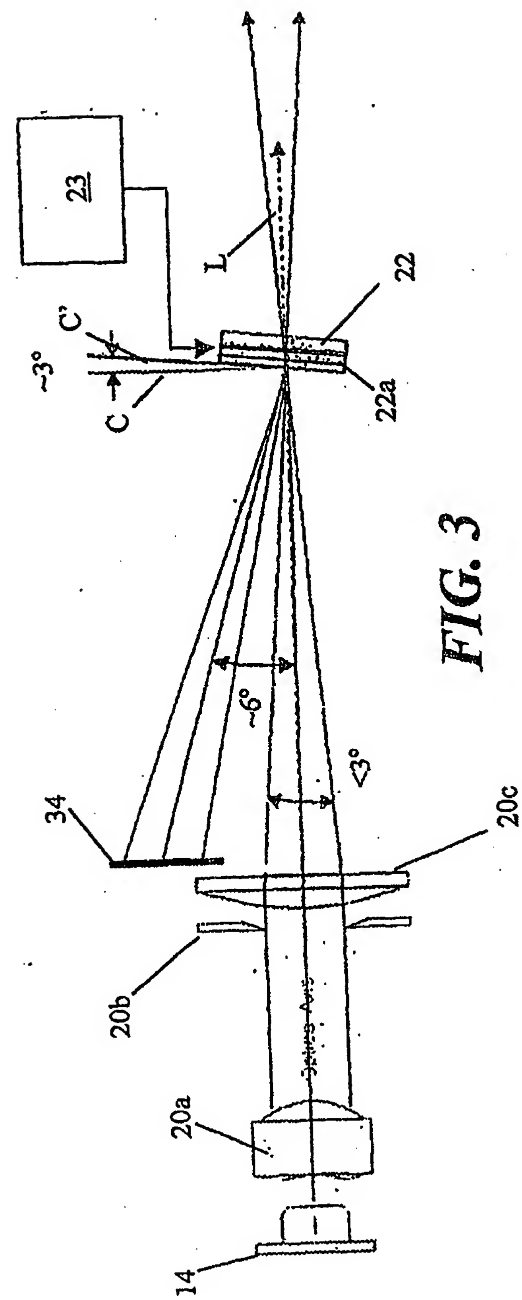
23
L
22
22a
C
C'
~3°
~6°
<3°
34
20c
20b
20a
14

FIG. 3